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Characterization of the Humoral Response Induced by a Synthetic Peptide of the Major Outer Membrane Protein of *Chlamydia trachomatis* Serovar B

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The major outer membrane protein of *Chlamydia trachomatis* has been extensively studied and is still considered one of the most promising candidates for development of a synthetic vaccine. Neutralizing epitopes in variable domains I, II, and IV have already been reported. In variable domain I, residues 69 to 78 have been identified as a neutralizing epitope for some of the C- and C-related complex serovars (A, C, I, J, L3, and K). It is not known whether epitopes located at the same position in B-complex serovars are neutralizing. To clarify this point, rabbit polyclonal antibodies directed against the peptide ^{69}TTTGNAVAPS^{78} from the B serovar were produced. Rabbit antisera were further rendered peptide specific by purification on a peptide-bovine serum albumin-Sepharose affinity column. Peptide-specific rabbit immunoglobulin reacted with five of the B-complex serovars (B, Ba, E, L1, and L2) by immunoblot and by direct-binding enzyme-linked immunosorbent assay. Furthermore, this peptide-specific rabbit immunoglobulin neutralized the chlamydial infectivity of both serovars B and E for HaK cells in a complement-independent in vitro assay. The importance of these results stems from the fact that peptide ^{69}TTTGNAVAPS^{78} was able to induce an antibody response directed against B- and B-related complex serovars, including serovar E, which is responsible for a high proportion of genital infections. This peptide could therefore be considered for the construction of a multivalent synthetic vaccine.

Chlamydia trachomatis is the leading cause of sexually transmitted diseases in industrialized countries and is also a major cause of preventable blindness in underdeveloped countries (13). On the basis of pathogenicity, antigenicity, and nucleic acid composition, human-pathogenic strains of *C. trachomatis* have been subdivided into 15 serovars (L1, L2, L3, A through K, and Ba). By their antigenic properties, these serovars were grouped into three complexes: the B complex (B, Ba, D, E, L1, and L2), the intermediate complex (F, G, K, and L3), and the C complex (A, C, H, I, and J) (7, 16). Intermediate serovars can also be subdivided into B-related (F and G) and C-related (K and L3) complex serovars.

All 15 serovars of *C. trachomatis* bear a major outer membrane protein (MOMP), which constitutes 60%, by weight, of the chlamydial outer membrane proteins (4). The MOMP genes encode a highly conserved protein structure that contains four evenly spaced domains, with sequences varying among the different serovars, called the four variable domains (VDs) (1, 14, 15). These VDs have been extensively studied, and neutralizing epitopes in VDs I, II, and IV have been reported (1, 8–11, 17, 22–24). Studies with synthetic peptides revealed that residues 69 to 78, located in VD I, correspond to a neutralizing epitope for some C and C-related serovars such as serovars A (1, 18, 19); C, I, J, and L3 (11, 24); and K (21). Such similarities in the locations of neutralizing epitopes among different serovars may suggest that this particular region of the MOMP, by both its exposure and charge, is highly immunogenic and could induce a protective response.

Previous studies of *C. trachomatis*-host cell interactions have suggested a role of adhesin for the MOMP (20). Indeed, negatively charged divergent sequences in exposed MOMP VDs are implicated in the binding of *C. trachomatis* to host cells via electrostatic interactions. This is consistent with the surface exposure and charge properties of the MOMP VDs of all serotypes, despite amino acid sequence variations. Thus, the fact that neutralizing epitopes within VD I of serovar C and C-related complex are found in the same position, despite amino acid sequence variations, suggests an intrinsic property of VD I. The present study aims to extend this finding to B-complex serovar VD I and to verify whether a synthetic peptide corresponding to residues 69 to 78 of the MOMP bears a neutralizing epitope.

The peptide used in the rabbit immunizations, ^{69}TTTG NAVAPS^{78}, named VB, corresponds to 10 amino acids of VD I of serovar B and was synthesized by the method of Geysen et al., using the recommendations supplied in the commercially available epitope mapping kit (5, 6). The peptide was conjugated to keyhole limpet hemocyanin with the EDC [1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride] procedure according to the manufacturer's recommendations (Pierce Chemical Co., Rockford, Ill.) and was used to produce rabbit polyclonal antisera. Two rabbits were injected subcutaneously with 500 μg of VB-keyhole limpet hemocyanin in complete Freund's adjuvant on day 0; this was followed by 500 μg of VB-keyhole limpet hemocyanin given intramuscularly in incomplete Freund's adjuvant at weeks 3, 6, 9, and 12. Animals were bled during weeks 5, 8, and 11 and sacrificed in week 14 of the immunization protocol. Sera were obtained and frozen at −80°C. Antipeptide titers were determined as previously described (3) by a direct-binding enzyme-linked immunosorbent assay (ELISA) on solid-phase-fixed peptides (50 μg/ml).

The antiserum with the highest titer was rendered peptide specific by affinity purification on a peptide-bovine serum albumin (BSA)-Sepharose column. Briefly, the peptide was

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first conjugated to BSA with the EDC procedure and then coupled to Sepharose CL4B. The antiserum was then passed through the column, and bound immunoglobulins (Ig) were eluted with glycine as previously described (2). Peptide-specific Ig were used for all assays.

Products of sodium dodecyl sulfate-polyacrylamide gel electrophoresis of the 15 *C. trachomatis* serovars, in 10% polyacrylamide gels under reducing conditions, were electrotransferred to nitrocellulose and used for immunoblotting (21).

Direct-binding assays were done by using heat-treated or non-heat-treated elementary bodies. Briefly, microtiter plates were coated overnight at 4°C with 100 μl of synthetic peptide (50 μg/ml) in carbonate-bicarbonate buffer (50 mM, pH 9.6) or with heat-treated (30 min, 56°C) or non-heat-treated elementary bodies (5 μg/ml) in phosphate-buffered solution (PBS) (pH 7.4). Peptide-specific Ig were diluted in PBS–0.5% BSA (5 μg/ml) and incubated at 37°C for 90 min. After washes, an anti-rabbit Ig–peroxidase conjugate was added, and the reaction was developed as described previously (21). The A_{405} was then measured. Statistical significance was evaluated with the Student *t* test.

The in vitro neutralization assay was performed as previously described (20). Briefly, purified elementary bodies were diluted in 0.25 M sucrose–10 mM sodium phosphate–5 mM L-glutamic acid (SPG) (pH 7.2) for a final concentration of 4×10^5 inclusion-forming units per ml. Bacteria were then mixed with an equal volume of monoclonal antibodies (MAbs) serially diluted in SPG–0.5% BSA. The mixture was incubated at 37°C for 60 min, and 400 μl of the appropriate *C. trachomatis*-antibody mixture dilutions was inoculated in triplicate onto confluent HaK cell monolayers without centrifugation. After 2 h of incubation, the inocula were removed and the monolayers were washed and fed with 500 μl of Eagle's minimal essential medium supplemented with 10% fetal calf serum containing 0.5 μg of cycloheximide per ml and incubated at 37°C for 72 h. The monolayers were fixed with methanol, and chlamydial inclusions were identified by indirect fluorescent-antibody staining with a genus-specific MAb to chlamydial lipopolysaccharide. The calculations for determining the percent reduction of inclusion-forming units by MAbs were done as described by Sabet et al. (12). A 50% or greater reduction from the number of control inclusion-forming units in infectivity was considered positive for neutralization.

We first produced a peptide-specific rabbit polyclonal antibody directed against an epitope of VD I of *C. trachomatis* serovar B. The fine specificity of rabbit antisera was then determined by a direct-binding ELISA on synthetic peptides. The significant antibody titers directed against the peptide ^{69}TTTGNAVAPS^{78} of serovar B suggest that this peptide is clearly immunogenic. Indeed, one anti-VB antiserum, obtained at week 14 of the immunization schedule, specifically reacted with the peptide ^{69}TTTGNAVAPS^{78} with a titer of 25,000, but it did not react with control peptides VA and VK, which are residues 69 to 78 of serovars A and K, respectively. Furthermore, this antiserum also reacted with whole *C. trachomatis* serovar B with a titer of 25,000, suggesting that the synthetic peptide mimics the natural conformation of the epitope. This peptide was used to purify antibodies by affinity chromatography.

The reactivity of peptide-specific Ig with the 15 *C. trachomatis* serovars was then tested. By immunoblot, it strongly reacted with five B-complex serovars (B, Ba, E, L1, and L2), but no reaction was noted with B-related, C-related, and C-complex serovars (Fig. 1). The same results were also obtained in a direct-binding ELISA using heat-treated or non-heat-treated serovars (Fig. 2). A lower reactivity against

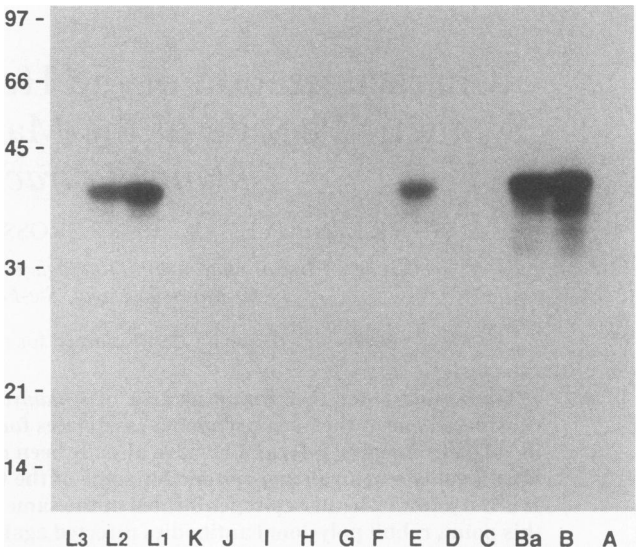

FIG. 1. Immunoblot profile of the peptide-specific rabbit Ig with the 15 serovars of *C. trachomatis*. Numbers on the left are molecular weights in thousands.

serovar E can be observed by direct-binding ELISA, and this correlates with results obtained by immunoblot. We cannot definitely explain these results, but we think that both substitution of threonine (residue 69) by serine in serovar E and substitution of serine (residue 78) by threonine could be involved in the lower reactivity. No significant differences were observed between reactions against heat-treated and non-heat-treated serovars, suggesting that the epitope ^{69}TTTGNAVAPS^{78} is surface exposed (Fig. 2).

Finally, the complement-independent in vitro neutralizing assay indicated that the specific antibody response induced by the peptide ^{69}TTTGNAVAPS^{78} was able to neutralize the

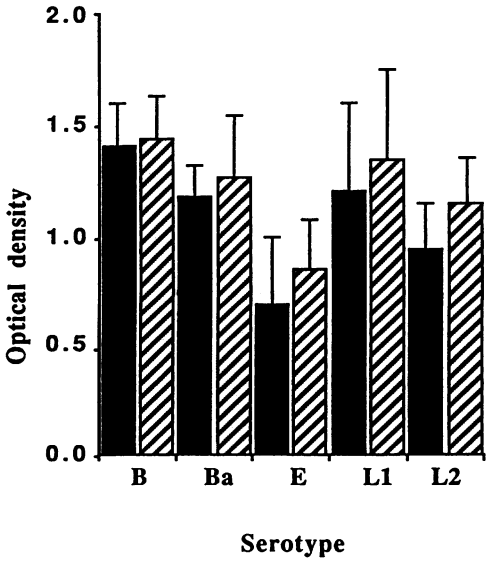

FIG. 2. Direct-binding ELISA of peptide-specific rabbit Ig with heat-treated (■) or non-heat-treated (▨) *C. trachomatis* serovars. Each number represents the mean value from three experiments; bars indicate standard deviations.

infectivities of serovars B and E. In fact, less than 20 μg of peptide-specific Ig per ml reduced infectivities of both serovars B and E by 50%, whereas control rabbit Ig had no effect, confirming that this antiserum could neutralize the infectivities of both serovars B and E. Although simple binding assays do not necessarily correlate to in vitro neutralization, it is tempting to speculate that this antibody will neutralize other serovars of the B complex. These experiments, including the production of homologous MAbs, are now in progress.

In summary, a number of investigators (1, 11, 18, 19, 24), including us (21), have reported the existence of neutralizing epitopes within VD I of the MOMP. The neutralizing epitope that we identified in VD I of serovar K is located in the same position (residues 69 to 78) as those reported for other serovars of C- and C-related complex (21). These data suggest that this region of the MOMP constitutes a neutralizing epitope despite amino acid sequence variations between serovars. In this study, we extended these data to serovar B, showing that a neutralizing epitope is found and located in the same position as reported for other serovars.

The results obtained with serovar B are of prime importance because they suggest that this particular region of VD I is not only a neutralizing epitope for the C and C-complex serovars, but also for the B- and B-related complex serovars. They also suggest that neutralization could be the result of a blockage by antibodies of the chlamydial attachment via inhibition of electrostatic interactions with host cells (20). Finally, it appears that the surface exposures of MOMP VDs, and not their primary sequences, determine their immunogenic properties. Consequently, the surface exposure of VD II raises the possibility that immunogenic and neutralizing properties of the MOMP from B and B-related serovars could also be located in this VD.

In conclusion, our synthetic peptide, which induces an antibody response against five of the 15 serovars of *C. trachomatis*, could be a good candidate for part of a multivalent synthetic vaccine.

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